

Gleiss & Große
Intellectual Property and Technology Law

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What is claimed is

1. An oligonucleotide that may be used as a primer, in particular a forward primer, to amplify a nucleic acid region of a genital human papilloma virus (HPV) and that has the sequence 5'-CAR GCI AAA WWW KTD AAR GAY TGT G-3' or 5'-CAR GCN AAA WWW KTD AAR GAY TGT G-3' (SEQ ID no. 1), wherein R = A or G, W = T or A, K = T or G, I = inosine, N = A, T, G, or C, D = A, T, or G, and Y = C or T.
2. The oligonucleotide of Claim 1, wherein the oligonucleotide is selected from the group comprising:
 - a) an oligonucleotide having the nucleotide sequence 5'-CAR GCI AAA TAT KTR AAA GAT TGT G-3' or 5'-CAR GCN AAA TAT KTR AAA GAT TGT G-3' (SEQ ID no. 2),
 - b) an oligonucleotide having the nucleotide sequence 5'-CAR GCA AAA TAT GTW AAG GAT TGT G-3' (SEQ ID no. 3),
 - c) an oligonucleotide having the nucleotide sequence 5'-CAR GCW AAA ATT GTA AAR GAT TGT G-3' (SEQ ID no. 4),
 - d) an oligonucleotide having the nucleotide sequence 5'-CAA GCA AAA ATA GTA AAR GAC TGT G-3' (SEQ ID no. 5), and

- e) an oligonucleotide having the nucleotide sequence 5'-CAR GCA AAA TAT GTA AAA GAC TGT G-3' (SEQ ID no. 6),

wherein R = A or G, W = T or A, K = T or G, I = is inosine, and N = A, T, G, or C.

3. An oligonucleotide that may be used as a primer, in particular a reverse primer, to amplify a nucleic acid region of a genital human papilloma virus having the nucleotide sequence 5'-ARY GGY TSY ARC CAA AAR TGR CT-3' (SEQ ID no. 7), wherein R = A or G, Y = C or T, and S = C or G.

4. An oligonucleotide of one of Claims 1 to 3 that has a nucleotide sequence that is mutated relative to one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, which may be obtained by the:

- a) deletion of 1 to 10 nucleotides in one of the nucleotide sequences recited in SEQ ID nos. 1 to 7,
- b) addition of 1 to 10 nucleotides in one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, and/or
- c) substitution of 1 to 3 nucleotides in one of the nucleotide sequences recited in SEQ ID nos. 1 to 7.

5. The oligonucleotide of Claim 4, wherein the deletion or addition of the nucleotides is present at the 5' and and/or 3' end of one of the nucleotide sequences recited in SEQ ID nos. 1 to 7.

6. The oligonucleotide of one of Claims 1 to 5, wherein said oligonucleotide is a DNA molecule, RNA molecule, PNA molecule, LNA molecule, or a hybrid form thereof.

7. The oligonucleotide of one of Claims 1 to 6, wherein the nucleotide sequence thereof is complementary to a sequence from the E1 gene region of at least one genital HPV genotype.

Gleiss & Große

8. An oligonucleotide that has a nucleic acid sequence that is complementary over its entire length to the nucleotide sequence of an oligonucleotide of one of Claims 1 to 7.

9. A primer pair for amplifying a nucleic acid region of a genital human papilloma virus (HPV) comprising a forward primer and a reverse primer, wherein the forward primer is selected from the group comprising:

- a) an oligonucleotide of Claim 1 or 2 having one of the nucleotide sequences recited in SEQ ID nos. 1 to 6,
- b) an oligonucleotide of Claim 4 or 5 having a nucleotide sequence that is mutated relative to the oligonucleotide of a), and
- c) a mixture of the oligonucleotides of a) and/or b),

and the reverse primer is selected from the group comprising:

- d) an oligonucleotide of Claim 3 having the nucleic acid sequence recited in SEQ ID no. 7,
- e) an oligonucleotide of Claim 4 or 5 having a nucleotide sequence that is mutated relative to the oligonucleotide of d), and
- f) a mixture of the oligonucleotides of d) and e).

10. The primer pair of Claim 9, wherein the forward primer is an equimolar mixture of the oligonucleotides having the nucleotide sequences recited in SEQ ID nos. 2 to 6, and the reverse primer is the oligonucleotide having the nucleotide sequence recited in SEQ ID no. 7.

11. A process for the amplification of a region of a nucleic acid of a genital human papilloma virus present in a biological sample comprising the implementation of a nucleic acid amplification process using a primer pair comprising a forward primer and a reverse primer, wherein the forward primer is selected from the group comprising:

Gleiss & Große

- a) an oligonucleotide of Claim 1 or 2 having one of the nucleotide sequences recited in SEQ ID nos. 1 to 6,
- b) an oligonucleotide of Claim 4 or 5 that has a nucleotide sequence that is mutated relative to the oligonucleotide of a), and
- c) a mixture of the oligonucleotides of a) and/or b),

and the reverse primer is selected from the group comprising:

- d) an oligonucleotide of Claim 3 having the oligonucleotide sequence recited in SEQ ID no. 7,
- e) an oligonucleotide of Claim 4 or 5 that has a nucleotide sequence that is mutated relative to the nucleotide of d), and
- f) a mixture of the oligonucleotides of d) and e).

12. The process of Claim 11, wherein the oligonucleotide is a DNA molecule, RNA molecule, DNA molecule, LNA molecule, or a hybrid form thereof.

13. The process of Claim 11 or 12, wherein the biological sample is a smear from the cervix, a fresh tissue sample, a fixed tissue sample, or a cross-sectional specimen of a tissue sample.

14. The process of one of Claims 11 to 13, wherein the nucleic acid that is to be amplified is purified and/or isolated from the biological sample.

15. The process of Claim 14, wherein the nucleic acid to be amplified is a DNA.

16. The process of one of Claims 11 to 15, wherein the nucleic acid amplification process is a PCR (polymerase chain reaction) process.

Gleiss & Große

17. The process of Claim 16, wherein the forward primer and the reverse primer in the nucleic acid amplification reaction are each used at a concentration of 0.5-1 pmoles/ μ L.

18. The process of Claim 17, wherein an equimolar mixture of the oligonucleotides having the nucleotide sequences recited in SEQ ID nos. 2 to 6 is used as the forward primer, wherein each oligonucleotide is present at a concentration of 0.1-0.2 pmoles/ μ L, and the oligonucleotide having the nucleotide sequence recited in SEQ ID no. 7 is used at a concentration of 0.5-1 pmoles/ μ L as the reverse primer.

19. The process of one of Claims 16 to 18, wherein the nucleic acid amplification is performed under the following temperature conditions:

- a) heat to 95°C, with the temperature increased by 1°C per sec,
- b) hold the temperature at 95°C for 10 min,
- c) perform 40 cycles, each comprising 30 sec at 95°C, 30 sec at 55°C, and 1 min at 72°C,
- d) hold the temperature at 72°C for 5 min, and
- e) cool to 4°C.

20. The process of one of Claims 11 to 15, wherein the nucleic acid amplification process is an LCR (ligase chain reaction) process, an NASBA process, or an isothermal process.

21. The process of one of Claims 11 to 20, wherein a region of the HPV gene E1 is amplified.

22. The process of one of Claims 11 to 21, wherein the amplified nucleic acid region is purified and/or isolated.

Gleiss & Große

23. The process of one of Claims 11 to 22, wherein the amplification product is provided with a mark during or after the amplification reaction.

24. A process for detecting and/or identifying a genital HPV genotype, comprising the testing of a nucleic acid of a genital human papilloma virus present in a biological sample, in particular the testing of the HPV gene E1 or a portion thereof, by hybridization with at least one probe, wherein the probe is selected from the group comprising:

- a) HPV genotype-specific oligonucleotides having the nucleotide sequences recited in SEQ ID nos. 8 to 135,
- b) oligonucleotides that have a nucleotide sequence that is mutated relative to one of the oligonucleotides of a), namely a deletion or addition of 1 to 10 nucleotides, or a substitution of 1 to 3 nucleotides in one of the nucleotide sequences recited in a),
- c) oligonucleotides that have a nucleotide sequence that is complementary over the entire length of the nucleotide sequence of an oligonucleotide of a) or b),
- d) nucleic acid molecules comprising at least one region that has one of the nucleotide sequences recited in a) to c) and one or more additional regions having a total length of at least one nucleotide, and
- e) mixtures of the oligonucleotides of a) to c) and/or of the nucleic acid molecules of d),

and the detection of the hybridization and wherein prior to the hybridization with the probe the HPV nucleic acid present in the biological sample is amplified, and the amplification of the nucleic acid is accomplished by a process of one of claims 11 to 23.

Gleiss & Große

25. The process of Claim 24, wherein the oligonucleotide or nucleic acid molecule that is used as a probe is a DNA molecule, RNA molecule, DNA molecule, LNA molecule, or a hybrid form thereof.

26. The process of one of Claims 24 or 25, wherein the biological sample is a smear specimen from the cervix, a fresh tissue sample, a fixed tissue sample, or a cross-sectional specimen of a tissue sample.

27. The process of one of Claims 11 to 26, wherein the process is used for the diagnosis and/or early detection of diseases, precursor stages of diseases, risks of diseases, and/or pathological changes caused by genital human papilloma viruses.

28. The process of Claim 27, wherein the disease is a cancer disease.

29. A nucleotide array for detecting and/or identifying the genotype of a human papilloma virus contained in a biological sample comprising a solid carrier having a surface and at least one first oligonucleotide or nucleic acid molecule bound to the carrier surface that is suitable for use as a probe for testing the HPV gene E1 or a portion thereof to detect and/or identify a genital human HPV genotype selected from the group comprising:

- a) HPV genotype-specific oligonucleotides having the nucleotide sequences recited in SEQ ID nos. 8 to 135,
- b) oligonucleotides that have a nucleotide sequence that is mutated relative to one of the oligonucleotides of a), namely, a deletion or addition of 1 to 10 nucleotides or a substitution of 1 to 3 nucleotides in one of the nucleotide sequences recited in a),
- c) oligonucleotides that have a nucleotide sequence that is complementary over its entire length to the nucleotide sequence of an oligonucleotide of a) or b) ,

Gleiss & Große

- d) nucleic acid molecules comprising at least one region that has one of the nucleotide sequences recited in a) to c) and one or more additional regions having a total length of at least one nucleotide, and
- e) mixtures of the oligonucleotides of a) to c) and/or of the nucleic acid molecules of d).

30. The nucleotide array of Claim 29, wherein the carrier is platelet-shaped, for example in the form of a microscope slide, or is platelet-shaped with depressions, for example as a chamber slide or as a microtiter plate having the dimensions stated in the recommendations of the SBS (Society of Biomolecular Screening).

31. The nucleotide array of Claim 29 or 30, wherein the first oligonucleotides or nucleic acid molecules on the surface of the carrier are located in a defined analysis area.

32. The nucleotide array of one of Claims 29 to 31, wherein the surface of the carrier has a control area.

33. The nucleotide array of Claim 32, wherein the control area comprises a control for orienting the carrier, an amplification control, a hybridization control, a sample control, and/or a print control.

34. The nucleotide array of Claim 33, wherein the control for orienting the carrier comprises at least one second oligonucleotide or nucleic acid molecule.

35. The nucleotide array of Claim 34, wherein the second oligonucleotide is a fluorescent oligonucleotide, and the control for orienting the carrier comprises at least three spots of the fluorescent oligonucleotide.

36. The nucleotide array of Claim 35, wherein the amplification control comprises at least one third oligonucleotide or nucleic acid molecule.

Gleiss & Große

37. The nucleotide array of Claim 36, wherein the third oligonucleotide or nucleic acid molecule is suitable for use as a probe for detecting an amplification product that is obtained by means of an amplification process using a control nucleic acid as the template and a primer pair of Claim 9 or 10.

38. The nucleotide array of Claim 37, wherein the control nucleic acid has a length and a GC content that corresponds to the length and the GC content of the amplification product that is obtained by means of an amplification process using the nucleic acid region of a genital human papilloma virus as the template and a primer pair of Claim 9 or 10.

39. The nucleotide array of Claim 33, wherein the hybridization control comprises at least one fourth oligonucleotide or nucleic acid molecule.

40. The nucleotide array of Claim 39, wherein the hybridization control comprises at least 2 to 10 spots of the fourth oligonucleotide or nucleic acid molecule, and the spots have variously defined amounts of the fourth oligonucleotide or nucleic acid molecule.

41. The nucleotide array of Claim 40, wherein the hybridization control comprises spots with a dilution series of the fourth oligonucleotide or nucleic acid molecule.

42. The nucleotide array of Claim 33, wherein the sample control comprises at least one fifth oligonucleotide or nucleic acid molecule.

43. The nucleotide array of Claim 42, wherein the fifth oligonucleotide or nucleic acid molecule is suitable for use as a probe for detecting the human ADAT1 gene.

44. The nucleotide array of Claim 33, wherein the print control comprises at least one sixth oligonucleotide or nucleic acid molecule.

45. Nucleotide array of one of Claims 29 to 44, wherein the oligonucleotides and nucleic acid molecules are embodied as DNA molecules, RNA molecules, PNA molecules, LNA molecules, or hybrid forms thereof.

Gleiss & Große

46. A kit for detecting and/or identifying genital HPV genotypes, comprising at least one first container having at least one primer for amplifying regions of the HPV gene E1, selected from oligonucleotides of one of Claims 1 to 3, oligonucleotides of Claim 4 or 5 having a nucleotide sequence that is mutated relative to one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, and/or primer pairs of Claim 9 or 10, and at least one second container having at least one probe for detecting an amplified region of the HPV gene E1, selected from oligonucleotides having one of the nucleotide sequences recited in SEQ ID nos. 8 to 135, having a mutated sequence thereof, oligonucleotides having a complementary sequence thereof, and nucleic acid molecules comprising at least one region having one of the nucleotide sequences recited in SEQ ID nos. 8 to 135, a mutated nucleotide sequence thereof, obtained through the deletion and/or addition of 1 to 10 nucleotides and/or substitution of 1 to 3 nucleotides of one of the nucleotide sequences recited in SEQ ID nos. 8 to 135, or a complementary nucleotide sequence thereof, and one or more additional regions having a total length of at least one nucleotide.

47. The kit of Claim 46, comprising at least 24 second containers having at least 24 different probes for detecting and/or identifying the HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35h, HPV39, HPV40, HPV42, HPV43, HPV44, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV59, HPV66, HPV68, HPV70, HPV73, and HPV82 genotypes, wherein each container contains at least one probe and wherein all probes contained in a container can detect only one specific genital HPV genotype.

48. A kit for detecting and/or identifying genital HPV genotypes, comprising at least one first container having at least one primer for amplifying regions of the HPV gene E1, selected from oligonucleotides of one of Claims 1 to 3, oligonucleotides of Claim 4 or 5 having a nucleotide sequence that is mutated relative to one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, and/or primer pairs of Claim 9 or 10, and a nucleotide array of one of Claims 29 to 45.

49. A kit of one of Claims 46 to 48, comprising two first containers, wherein one container contains equimolar amounts of the oligonucleotides having the nucleotide

sequences recited in SEQ ID nos. 2 to 6, or mutated sequences thereof, and one container contains an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 7 or a mutated nucleotide sequence thereof.

50. A kit of one of Claims 46 to 48, comprising six first containers, wherein five containers each contain one of the oligonucleotides having the nucleotide sequences recited in SEQ ID nos. 2 to 6, or mutated sequences thereof, and one container contains an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 7, or a mutated nucleotide sequence thereof.

51. A kit of one of Claims 46 to 50, additionally comprising a container having a control nucleic acid that may be amplified using an oligonucleotide of Claim 1 or 2 with one of the nucleotide sequences recited in SEQ ID nos. 1 to 6 as the forward primer, and using an oligonucleotide of Claim 3 having the nucleotide sequence recited in SEQ ID no. 7 as the reverse primer.

52. The use of an oligonucleotide of one of Claims 1 to 3, of an oligonucleotide of Claim 4 or 5, whose nucleotide sequence is mutated relative to one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, or of a primer pair of Claim 9 or 10 for amplifying a nucleic acid region of a genital human papilloma virus.

53. The use of an oligonucleotide having one of the nucleotide sequences recited in SEQ ID nos. 1 to 135, of an oligonucleotide whose nucleotide sequence is mutated relative to one of the nucleotide sequences recited in SEQ ID nos. 1 to 135, of an oligonucleotide that has a nucleotide sequence that is complementary to one of the nucleotide sequences recited in SEQ ID nos. 8 to 135, or to a mutated sequence thereof, of a nucleic acid molecule that comprises one of the nucleotide sequences recited in SEQ ID nos. 8 to 135, a mutated sequence thereof, or a complementary sequence thereof, or of a primer pair of one of Claims 9 or 10, for the diagnosis and/or early detection of diseases caused by genital human papilloma viruses.

Gleiss & Große

54. The use of an oligonucleotide having one of the nucleotide sequences recited in SEQ ID nos. 1 to 135, in particular of one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, 19, 32, 41, 44, 48, 82, and 117 to 135, of an oligonucleotide whose nucleotide sequence is mutated relative to one of the nucleotide sequences recited in SEQ ID nos. 1 to 135, in particular to one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, 19, 32, 41, 44, 48, 82, and 117 to 135, of an oligonucleotide that has a nucleotide sequence that is complementary to one of the nucleotide sequences recited in SEQ ID nos. 8 [sic] to 135, in particular to one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, 19, 32, 41, 44, 48, 82, and 117 to 135, or a mutated sequence thereof, of a nucleic acid molecule that comprises one of the nucleotide sequences recited in SEQ ID nos. 8 to 135, in particular one of the nucleotide sequences recited in SEQ ID nos. 19, 32, 41, 44, 48, 82, and 117 to 135, a mutated sequence thereof, or a complementary sequence thereof, or of a primer pair of one of Claims 9 or 10 to prepare a means for the diagnosis of diseases that are caused by genital human papilloma viruses.

55. The use of Claim 54, wherein the means is a nucleotide array of one of Claims 29 to 45.

56. The use Claim 54, wherein the diagnostic means is a kit of one of Claims 46 to 51.

57. The use of one of Claims 52 to 56, wherein the oligonucleotide or nucleic acid molecule is a DNA molecule, RNA molecule, PNA molecule, LNA molecule, or a hybrid form thereof.